

Full Length Research Paper

***In vitro* cytotoxic potential of *Swietenia macrophylla* King seeds against human carcinoma cell lines**

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Fruits of *Swietenia macrophylla* King (sky fruit) have been widely used in traditional medicine to treat various diseases including hypertension and diarrhea. The cytotoxic activity of the crude ethanol extract of the seeds of *S. macrophylla* and its fractions was assessed against selected human cancer cell lines, namely, HCT 116 (colon carcinoma), KB (nasopharyngeal epidermoid carcinoma), Ca Ski (cervical carcinoma) and MCF-7 (breast carcinoma) by using MTT assay. The *S. macrophylla* ethyl acetate fraction (SMEAF) showed the most potent activity against HCT116 cell line ($IC_{50} = 35.35 \mu\text{g/ml} \pm 0.50 \mu\text{g/ml}$) in a dose- and time-dependent manner and was further investigated for its possible mechanisms using flow cytometric analysis. Annexin V and PI binding of treated HCT116 cells indicated apoptosis induction by SMEAF in a dose-dependent manner. The induction of apoptosis was further confirmed both by DNA fragmentation using TUNEL assay and the externalization of phosphatidylserine using Annexin V/PI stain. The cell cycle analysis revealed a prominent increase in sub-G1 population at concentrations of 0.05 mg/ml and above. Results also showed that SMEAF induced collapse of the mitochondrial membrane potential after 24 h and caused depletion in total intracellular glutathione. The result of the present investigation is the first report on the potential anticancer activity of *S. macrophylla* seeds and its possible mechanisms of action on cancer cell proliferation.

Key words: *Swietenia macrophylla*, sky fruit, cytotoxicity, apoptosis.

INTRODUCTION

Plant-derived compounds have always been an important source of medicines for various diseases and have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxicity and cancer chemopreventive effects (Gonzales and Valerio, 2006). Recently there is considerable scientific and commercial interest in the continuing discovery of novel anticancer agents of plant origin (Cragg and Newman, 2005) and such investigations targeting plant products have recently regained prominence with the increasing understanding of their biological significance and increasing recognition

of the origin and function of their structural diversity (Conforti et al., 2008). Cancer is the second leading cause of death and its incidence has increased dramatically worldwide (Madhusudan and Middleton, 2005). Cancer is the uncontrolled growth and spread of abnormal cells, associated with dysregulation of apoptosis, a programmed cell death. Most of the current anticancer drugs are derived from plant sources, which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity.

The plant *Swietenia macrophylla* (Family: Meliaceae), commonly known as "sky fruit", because its fruits seem to point upwards to the sky, is a beautiful, lofty, evergreen large tree usually 30-40m in height and 3-4m in girth. This tropical timber tree grows natively throughout the tropical region of the America, especially in Central

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America, Mexico and Bolivia. In Malaysia, the seeds are used traditionally to treat diabetes, hypertension and also to relieve pain.

The seeds of *S. macrophylla* have been previously reported to have, anti-inflammatory, anti-mutagenicity and anti-tumor activity (Guevera et al., 1996), anti malaria (Soediro et al., 1990), anti-microbial (Maiti et al., 2007), *in vivo* anti-diarrheal activity (Mandal et al., 2007), and anti-nociceptive activity (Das et al., 2009). The seeds have been used commercially in healthcare products to improve blood circulation and skin condition. Phytochemical investigation has identified limonoids and its derivatives as the major constituents of this plant (Chen et al., 2009). The limonoid compounds that have been isolated are swietenine, swietenolide (Sircar and Chakraborty, 1951), Diacetylswietenolide, 6-O-acetylswietenolide (Goh et al., 2010a, b), swietemahonin, khayasin, andirobin, augustineolide, 7-deacetoxy-7-oxogedunin, proceranolide, and 3 β ,6-dihydroxydihydrocarapin (Mootoo et al., 1999). These limonoids were claimed to be responsible for its bioactivities.

To the best of our knowledge, the effect of *S. macrophylla* seeds on human cancer cell lines *in vitro* has hitherto not been reported. Thus, in the current study, we aimed to investigate the cytotoxic activity of *S. macrophylla* seeds and elucidate the possible mechanisms underlying the cytotoxic effect in human cancer cell lines.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used were analytical grade. Ethanol, hexane, ethyl acetate, hexane, dimethyl sulfoxide (DMSO) were purchased from Merck. RPMi 1640 medium, Sodium bicarbonate, HEPES, MTT, phosphate buffer saline (PBS) were supplied by Sigma-Aldrich while accutase was supplied by Innovative Cell Technologies, Inc. Foetal bovine serum (FBS), penicillin/streptomycin (100x) and amphotericin B (250 μ g/ml) were sold by PAA Laboratories. HCT 116 (colon carcinoma), KB (nasopharyngeal epidermoid carcinoma), Ca Ski (cervical carcinoma), MCF-7 (breast carcinoma) and Hep G2 (hepatocellular carcinoma) cell lines were obtained from American type culture company (ATCC).

Plant material

The dried seeds of the *S. macrophylla* (600 g) were purchased from local market and were identified and a voucher specimen (No. KLU046901) is on deposit at Herbarium of Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia.

Extraction procedures

Ground sample (600 g) was soaked in ethanol for 72 h with occasional stirring at room temperature. The extracting solvent was decanted and concentrated with a rotary vacuum evaporator at

40°C. A small portion of the crude ethanol extract (about 2.0 g) was stored in a freezer for bioassay screening while the balance of the extract was extracted with hexane repeatedly. The extracting solvents were combined, dried with anhydrous sodium sulphate, filtered and concentrated with a rotary vacuum evaporator. The hexane insoluble residue was then subjected to solvent-solvent partitioning using ethyl acetate and water in the ratio of 1:1. Both layers were later separated, filtered and evaporated to dryness to obtain ethyl acetate and aqueous fractions. Finally, the crude ethanol extract and its fractions (hexane, ethyl acetate and aqueous) were subjected for screening of various activities.

Cell culture

HCT 116, Ca Ski, MCF-7 and KB cell lines were cultured in RPMi 1640 while Hep G2 cell line in DMEM (with glutamine) supplemented with 10% heat-inactivated Fetal bovine serum, 50 IU/ml penicillin, 50 μ g/ml streptomycin and 0.25 μ g/ml amphotericin B. Cells were maintained at 37°C in 5% CO₂ atmosphere with 95% humidity.

MTT assay

The effect of *S. macrophylla* extracts on cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to Mosmann (1983) with some modifications. Cells were seeded at a density of 5×10^3 cells/well into a sterile flat bottom 96-well plate and allowed to adhere overnight. 20 μ l of the appropriate extract solution in the concentration range of 0 to 200 μ g/ml was then added. Cells were incubated with the extract for 72 h. 20 μ l of MTT (Sigma, filter sterile, 5 mg/ml) was then added to each well and the plates were incubated at 37°C in a humid atmosphere with 5% CO₂, 95% air for 4 h. The medium was then gently aspirated, and 150 μ l of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The final concentration of DMSO was 0.5% (v/v) and this was incorporated as a negative control in all experiments. The amount of formazan product was measured spectrophotometrically at 570 and 650 nm as a background using a (Oasys UVM340) microplate reader. The percentage of viability = (absorbance of treated cells/absorbance of untreated cell) x 100%.

Total glutathione (GSH) assay

The assay started by seeding 1×10^6 of cells into 23 cm² disks and incubated in 37°C for 24 h. After the 24 h of incubation period, the medium is replaced with the new medium containing different concentration of extracts ranging from 50 μ g/ml to 1 mg/ml. The disks were further incubated either for 24 h and harvested and lysed. The lysate was subjected to 10,000 g centrifugation. 10 μ l of the supernatant was loaded into each well and 150 μ l of working solution (8 ml buffer, 228 μ l of GR enzyme concentration 6 unit/ml and 228 μ l of DTNB 1.5 mg/ml) was then added and left for 5 min before 50 μ l of NADPH solution (0.16 mg/ml) was added into the wells. Once the NADPH solution was added, the absorbance values at 412 nm were measured immediately for 10 min continuously at one minute intervals with an Oasys UVM340 microplate reader and compared with glutathione standard curve. The results were expressed in nmoles of glutathione.

Mitochondrial membrane potential analysis

JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) is the dye used to

signal the loss of mitochondrial membrane potential (Reers et al., 1995). In healthy non-apoptotic cells, the dye stains the mitochondria bright red (Cossarizza et al., 1993). Conversely, the mitochondrial membrane potential collapsed in apoptotic cells and JC-1 cannot accumulate within the mitochondria and this caused JC-1 to remain in cytoplasm as green fluorescent monomeric form (Cossarizza et al., 1993). After the exposure to the sample and centrifugation, the cell pellets were resuspended in JC-1 solution and further incubated at 37°C in the CO₂ incubator for 15 min. Cells were then washed twice with fresh media and resuspended in fresh medium. analysis was carried out using a FACSCalibur flow cytometer and CellQuest software.

Cell cycle analysis

DNA content and cell cycle distribution were assessed using PI staining. After sample treatment, both adherent and floating cells were harvested, washed with phosphate buffered saline (PBS) and fixed with ice-cold absolute ethanol at -20°C overnight. Fixed cells were washed and resuspended in a buffer containing 50 µg/ml PI, 0.1% sodium citrate, 0.1% Triton- X-100 and 100 µg/ml of RNase A and incubated for 1 h in the dark at room temperature. PI stained cells were then analyzed by FACSCalibur flow cytometer (Becton Dickinson). Apoptotic cells were considered to constitute the sub-G1 population and the percentages of non-apoptotic cells in each phase of the cell cycle were determined.

Annexin V/PI

Briefly, after treatment, both floating and attached cells were harvested, washed with PBS twice, resuspended in annexin V binding buffer (BD Pharmingen) and stained with annexin V-FITC and PI (BD Pharmingen) for 30 min in the dark at room temperature. Data acquisition was carried out on a FACSCalibur flow cytometer (Becton Dickinson) and analyzed using CellQuest software (BD Biosciences). Annexin V can detect both the early and late stages of apoptosis and PI can detect late apoptosis and necrosis. The discrimination between viable (both annexin V and PI negative), early apoptotic (annexin V positive and PI negative), late apoptotic (both annexin V and PI positive) and necrotic (annexin V negative and PI positive) cells could be achieved by quantitatively estimating the relative amount of the annexin V/PI-stained cells in the population.

TUNEL assay

For detection of DNA fragmentation, TUNEL assay kit from Sigma was used. Treated-cells was harvested, washed with PBS and fixed with 1% (w/v) paraformaldehyde in PBS on ice for 15 min. After fixation, the cells were centrifuged, washed with PBS and incubated in DNA labeling solution provided by the kit for 60 min at 37°C. At the end of the incubation time, the cells were washed with rinsing buffer twice followed by incubation with antibody solution for 30 min at room temperature. After incubation, 0.5 ml of the propidium iodide/RNase A solution was added to cells and further incubated for 30 min in the dark at room temperature. The cells was analyzed using a FACSCalibur flow cytometer.

Statistical analysis

The data were expressed as mean \pm S.E. of three independent experiments or mean \pm S.D. of three replicates.

RESULTS

Cytotoxic effect of *S. macrophylla* on different cancer cell lines

Figures 1A-E show the results of cytotoxicity screening of ethanol extract and fractions against five different human cancer cell lines (HCT 116, Ca Ski, KB, MCF-7 and Hep G2) with increasing extract concentrations in the range of 10 to 200 µg/ml. The IC₅₀ values are shown in Table 1. Among the five different cell lines *S. macrophylla* ethyl acetate fraction (SMEAF) showed selective cytotoxic effect against HCT116 cells with IC₅₀ value of 35.35 µg/ml at 72 h of incubation period. The effect of SMEAF on cell viability was further evaluated at 24 and 48 h by determining the percentage of MTT reduction upon incubation of HCT116 cells and the results are shown in Figure 1F. The values of IC₅₀ decreased from 156.30 \pm 10.25, 64.05 \pm 7.45 to 35.35 \pm 0.50 µg/ml after incubation for 24, 48 and 72 h, respectively. Results showed that ethyl acetate fraction significantly reduced viability of cells in time- and dose-dependent manner.

Externalization of phosphatidylserine

Apoptosis is an active form of programmed cell death with several biochemical features (Jacobson et al., 1997; Nagata, 1997) and membrane-bound apoptotic bodies (Kidd, 1998). Transverse redistribution of plasma membrane phosphatidylserine (PS) occurs during early apoptosis; thus, the annexin V binding assay was performed to detect the surface exposure of PS. Figure 2A shows the FACS histograms with dual parameters including V-FITC and PI for different concentration of treatment (24 h); Figure 2B shows different hours of incubation period 24, 48 and 72 h for 0.05 mg/ml of ethyl acetate fraction. The dual parametric dot plots combining annexin V-FITC and PI fluorescence showed the viable cell population in the lower left quadrant (annexin V-negative/PI-negative), the early apoptotic cells in the lower right quadrant (annexin V-positive/PI-negative), and the late apoptotic cells in the upper right quadrant (annexin V-positive/ PI-positive). In untreated HCT116 cells, 3.23% of cells were the early apoptotic cells, 6.20% were late apoptotic cells. The early and late apoptotic cells increased to 4.95 and 18.96%, respectively, after being treated with 1.0 mg/ml of ethyl acetate fraction 24 h. Figure 2B shows significant increase of percentage of late apoptotic and dead cells throughout the increasing incubation period, with decreasing percentage of early apoptotic cells.

Induction of DNA fragmentation detected by TUNEL assay

The induction of apoptosis was further studied and quantified by performing terminal deoxynucleotidyl

Table 1. IC₅₀ values ($\mu\text{g/ml}$) of extract and fractions from *S. macrophylla* seeds against different cell lines, calculated after 72 h exposure.

Cell Lines	IC ₅₀ ($\mu\text{g/ml}$)			
	Ethanol	Hexane	Ethyl acetate	Aqueous
MCF-7	88.43 \pm 1.17	>200	116.43 \pm 3.18	>200
KB	106.72 \pm 6.9	>200	94.29 \pm 2.18	>200
HepG2	135.49 \pm 4.01	>200	92.52 \pm 0.53	>200
CasKi	115.89 \pm 4.51	>200	170.08 \pm 6.96	>200
HCT116	48.27 \pm 1.65	>200	35.35 \pm 0.50	>200

The data represent mean \pm S.E. of three independent experiments (n=9).

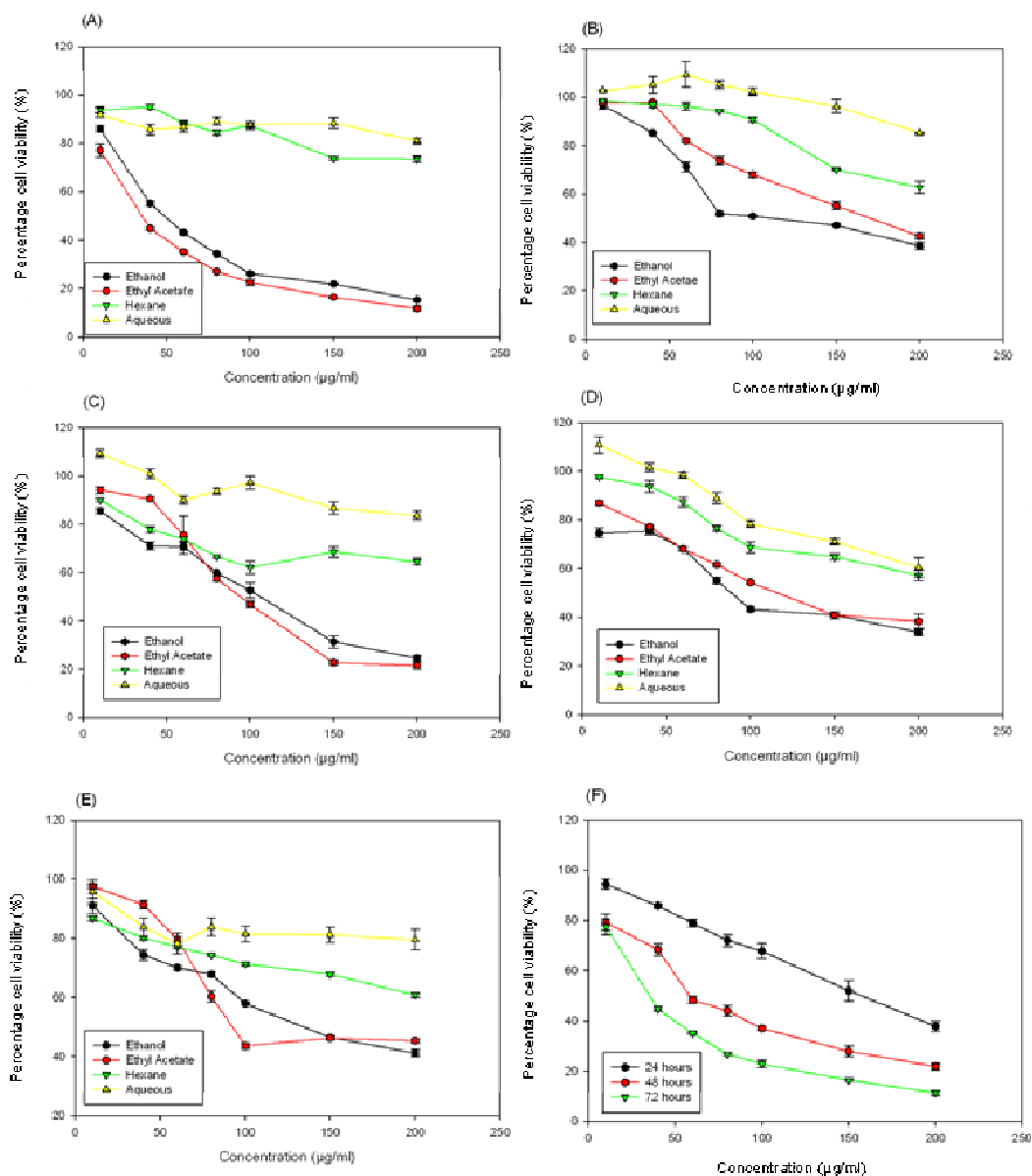
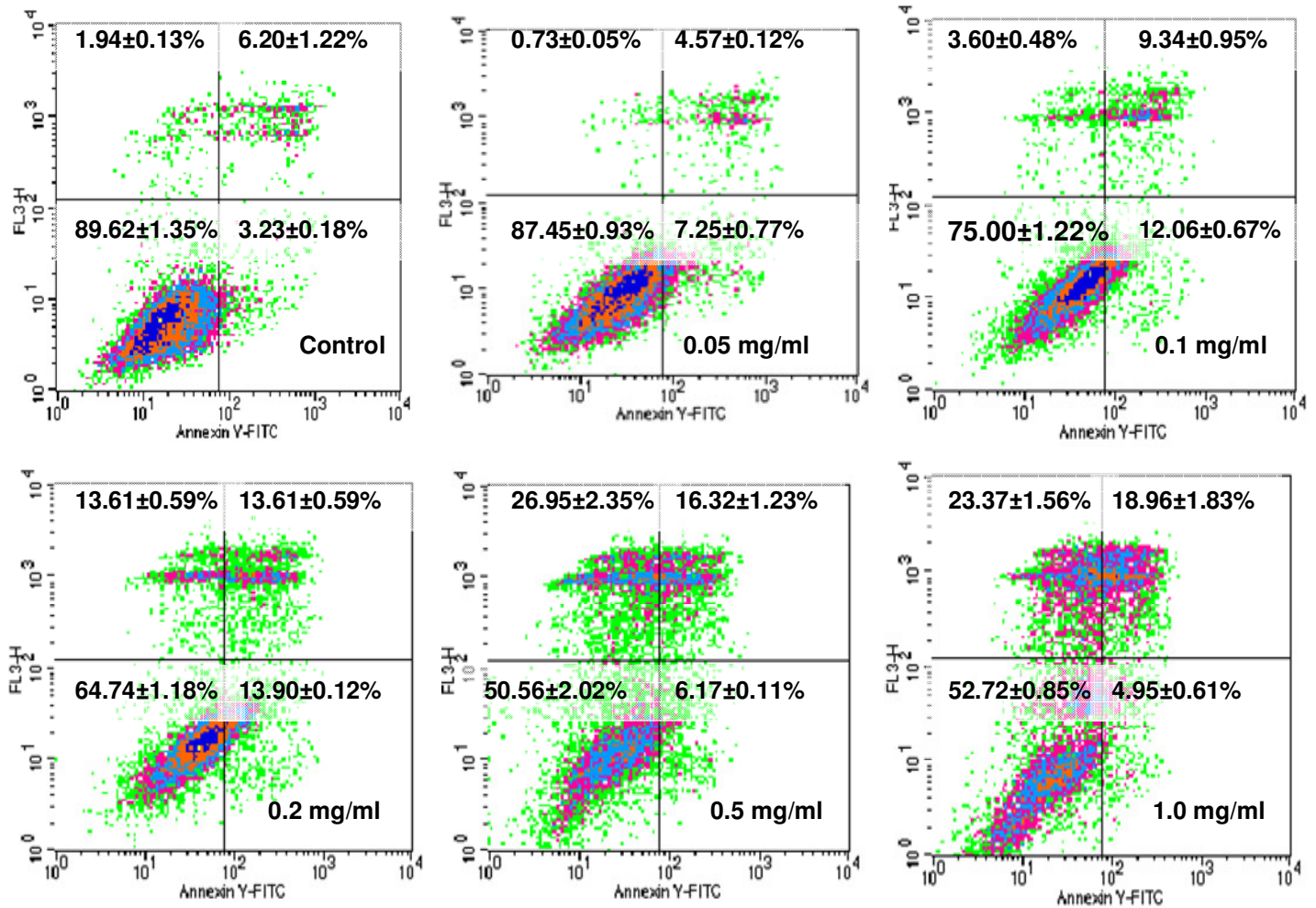
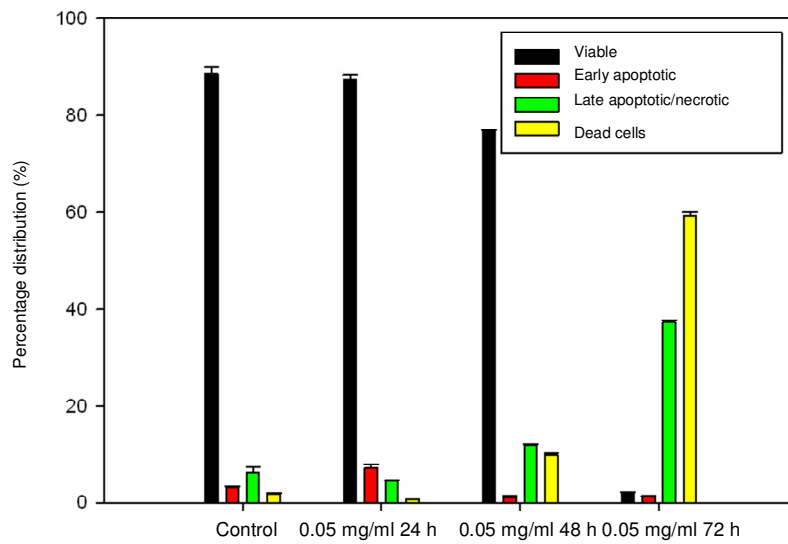


Figure 1. The cytotoxicity effect of *S. macrophylla* ethanol extract and fractions against various cancer cell lines at 72h incubation time. The measurements were taken using MTT cell viability assay. The graphs in 1A – E represent the cytotoxicity effects against HCT 116, Ca Ski, KB, MCF-7 and Hep G2, respectively. Graph 1F represent the cytotoxic effect of *S. macrophylla* ethyl acetate fraction on HCT116 at 24, 48 and 72 h of incubation period. The data represent mean \pm S.E. of three independent experiments (n=9).



A



B

Figure 2. Effect of *S. macrophylla* ethyl acetate fraction on the induction of phosphatidylserine externalization and cell membrane integrity in HCT116 cells undergoing apoptosis, measured using flow cytometry analysis. Histogram in (A) shows induction with dual parameters including V-FITC and PI for different concentrations of treatment at 24 h; Histogram in (B) shows induction at different incubation periods of 24, 48 and 72h for 0.05mg/ml of ethyl acetate fraction. The data represent mean±S.D. of three replicates. (n=3).

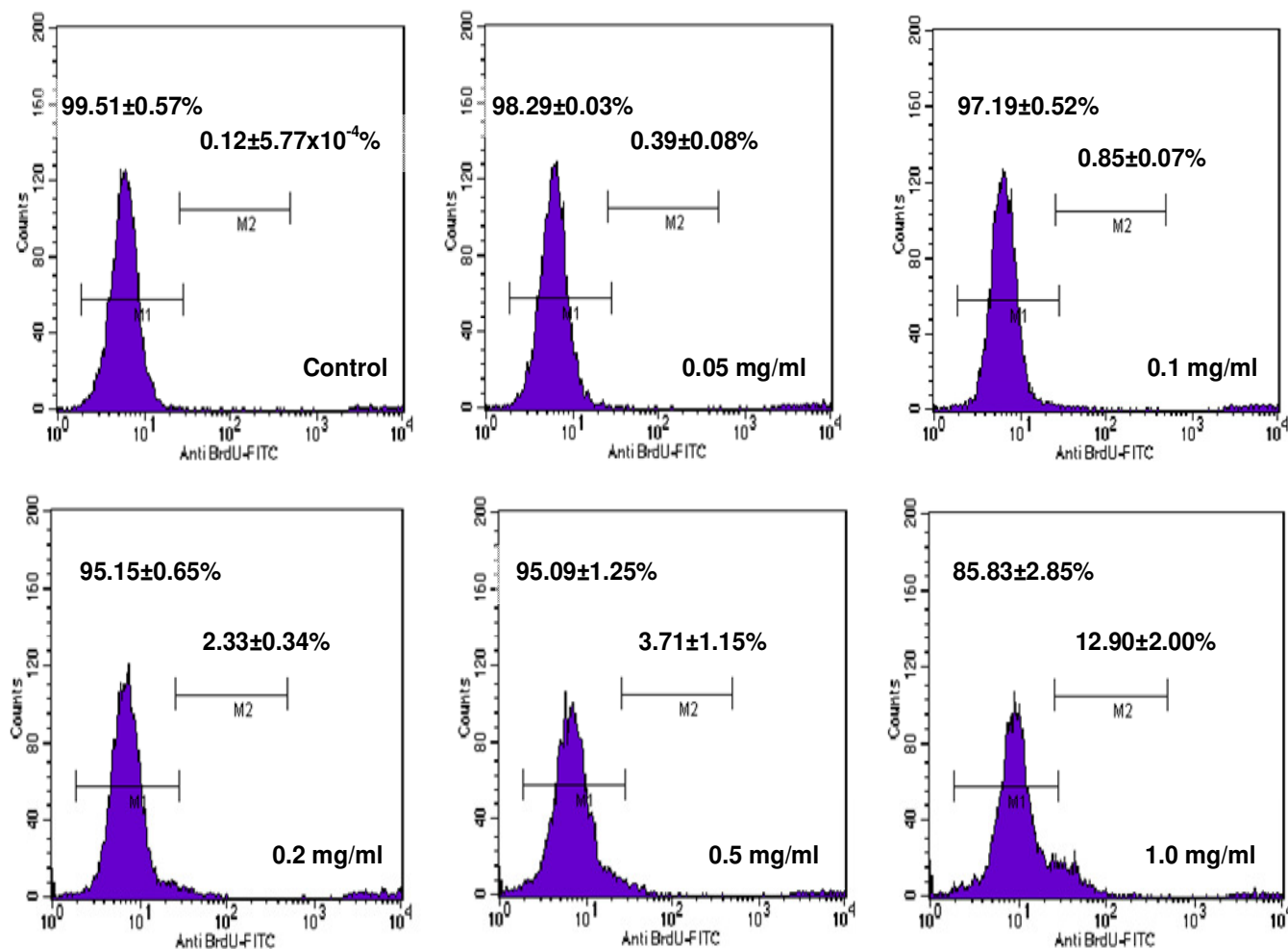


Figure 3. Effect of *S. macrophylla* ethyl acetate fraction on DNA fragmentation of HCT116 cells, measured by TUNEL assay using flow cytometry analysis, at increasing concentrations ranging from 0.05 mg/ml to 1mg/ml, 24 h following treatment. The data represent mean ± S.D. of three replicates (n=3).

transferase-mediated d-UTP Nick End Labelling, utilizing APO-BRDU kit. DNA breakage that occurred during late apoptosis was observed in Figure 3 after treatment of ethyl acetate fraction. The apoptotic index was 0.12% in control cells, and it increased to 0.39, 0.85, 2.33, 3.71 and 12.90% in cells treated with increasing concentrations ranging from 0.05 to 1.0 mg/ml.

Cell cycle distribution analysis

To determine whether the HCT116 cells treated with SMEAF undergo the apoptosis accompanied by alteration in the cell cycle, the distribution index was examined by PI staining. The increasing accumulation of G1 phase of cell distribution was observed as low as concentration of 0.05 mg/ml, and increased toward 1.0 mg/ml for 24 h incubation period (Figure 4A). This was accompanied by an increase of sub-G1 population and

concurrent decrease of G2/M phase cells with increasing hours of incubation at 0.05 mg/ml as shown in Figure 4B.

Alteration of intracellular total glutathione (GSH) level

Impairment of mitochondrial function may lead to a decrease in cytosolic glutathione, as GSH synthesis required ATP and deficiency of energy supplied by mitochondria is likely to affect cellular turnover of GSH (Mithöfer et al., 1992). Eventually, changes in the level of glutathione in cells might eventually lead to apoptosis (Ratan et al., 1994). Figure 5 shows the glutathione level which was obtained from the test. The results showed a significant dose-dependency in the drop of GSH level corresponding to the increase of ethyl acetate fraction concentrations. The lowest GSH level was established at 9.56 ± 0.50 nmoles when treated with 1.0 mg/ml of ethyl acetate fraction.

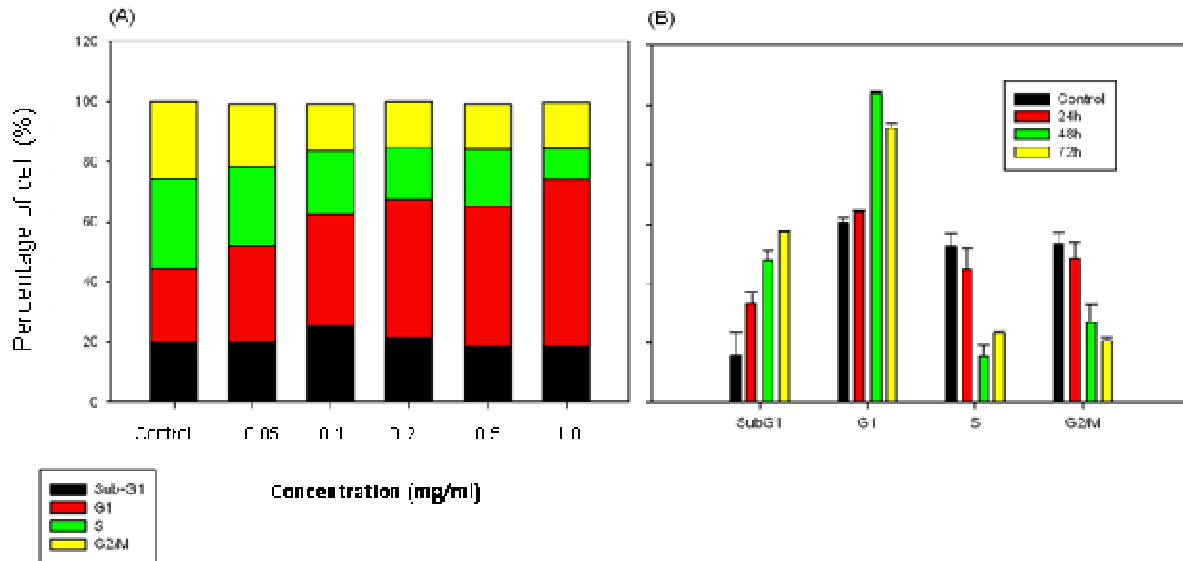


Figure 4. Effect of *S. macrophylla* ethyl acetate fraction on DNA distribution patterns of HCT116 cells. Propidium iodide (PI) fluorescent intensity was measured using flow cytometry. (A) DNA distribution in different phases of cells following treatments ranging from 0.05mg/ml to 1.0mg/ml for 24-hour incubation period. (B) DNA distribution in different phases of cells following treatment of 0.05mg/ml at 24, 48 and 72 h incubation periods. The data represent mean \pm S.D. of three replicates (n=3).

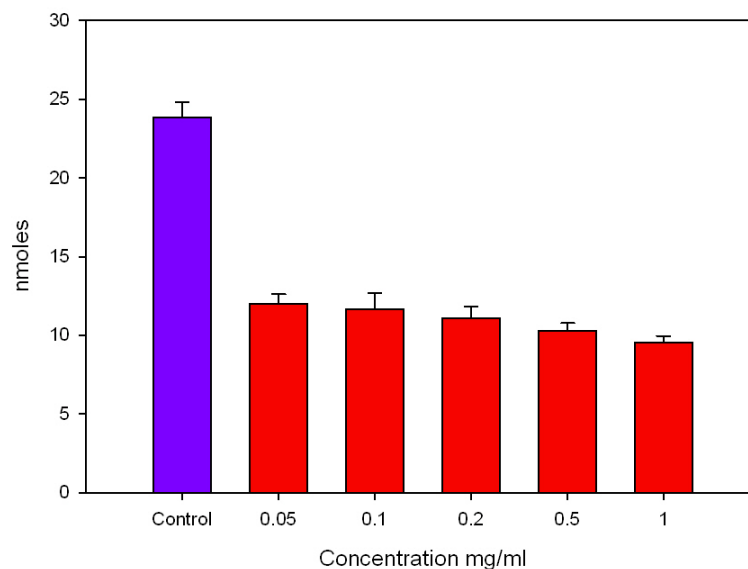


Figure 5. Effect of *S. macrophylla* ethyl acetate fraction on intracellular total glutathione level of HCT116 cells at 24 h following treatment. The data represent mean \pm S.E. of three independent experiments (n=9).

Disruption of mitochondrial membrane potential ($\Delta\Psi_m$)

Mitochondria play an important role in apoptotic cascade by serving as a convergent center of apoptotic signals originating from both the extrinsic and intrinsic pathways

(Kim et al., 2006). Depletion of $\Delta\Psi_m$ caused the opening of the mitochondria permeability transition pore, eventually leading to apoptosis (Narita et al., 1998). The changes in mitochondrial membrane potential in HCT116 cell line with ethyl acetate fraction were measured by JC-1 dye. Results in Figure 6 showed that most of the JC-1

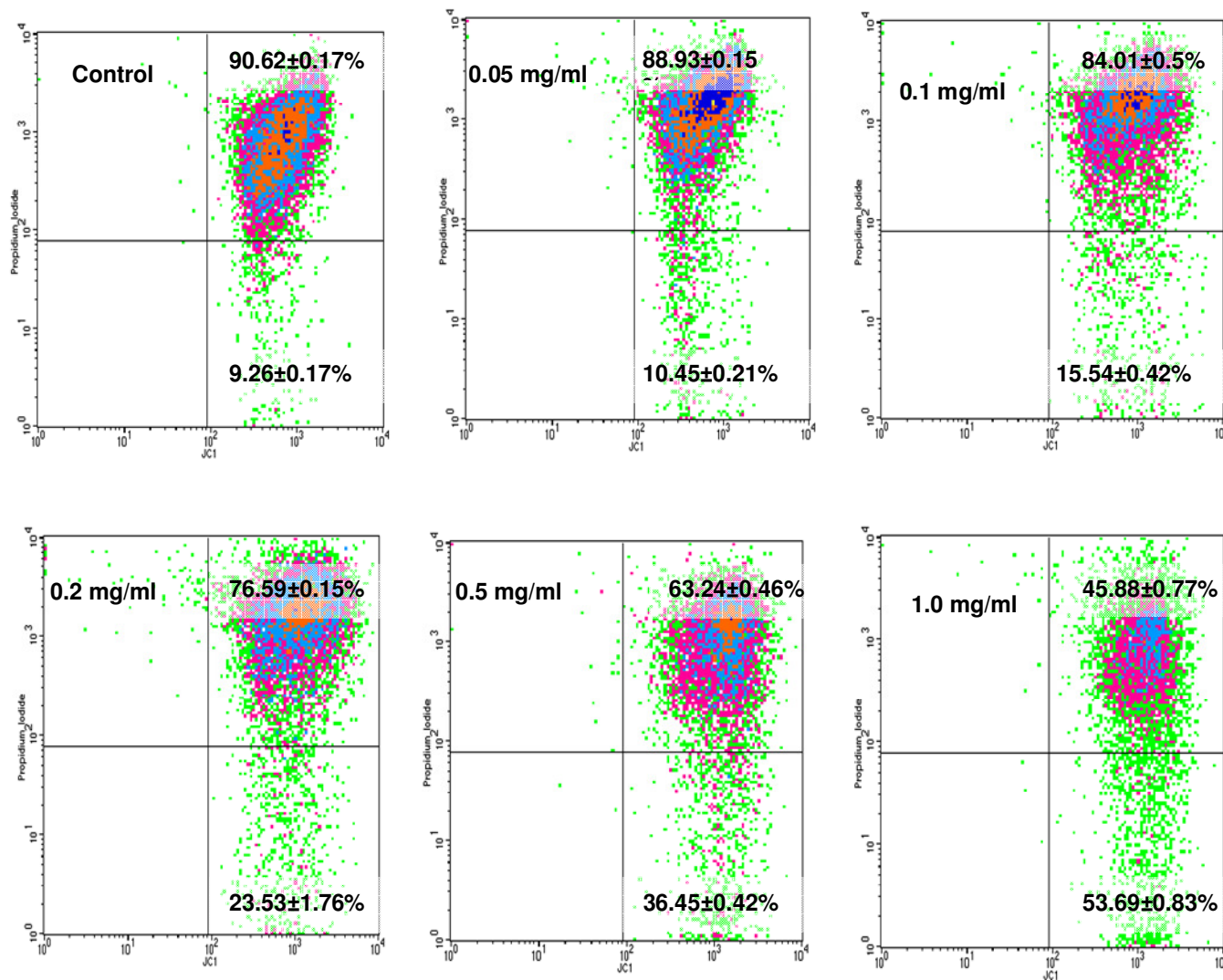


Figure 6. Effect of *S. macrophylla* ethyl acetate fraction on the mitochondrial membrane depolarization of HCT116 cells at various concentrations ranging from 0.05 to 1.0 mg/ml. JC-1 intensity was measured using flow cytometry fluorescence pattern analysis. The data represent mean \pm S.D. of three replicates (n=3).

fluorescence in the upper right quadrant of control untreated cells moved to the lower right quadrant after the treatment. The results indicated that the treatment has caused the depletion of $\Delta\Psi_m$ in a dose-dependent manner.

DISCUSSION

One of the goals of anticancer potential of any drug/extract is the induction of apoptosis in cancer cells (Denicourt and Dowdy, 2004). Apoptosis or programmed cell death is one of the most important targets for cancer treatment. It is characterized by membrane blebbing, cytoplasmic condensation, formation of apoptotic bodies, DNA fragmentation, alteration in membrane symmetry,

activation of cascade of caspases, and loss of mitochondrial membrane potential (Kang et al., 2007). In the present study, cytotoxic potential of SMEAF was assessed by MTT assay against cancer cell lines (HCT 116, KB, Ca Ski and MCF-7 and HepG₂). Assay is based upon reduction of yellow tetrazolium salt (MTT) by the reductase enzyme in metabolically active cells to a dark blue formazan (Van et al., 1994), which has been employed by many workers to measure cytotoxicity to cells.

Apart from the bark and leaf extracts initial screening on KB cells (Camacho et al., 2003), to the best of our knowledge, this is the first study to show that *S. macrophylla* seeds were able to cause cytotoxicity towards cancer cells and induced apoptosis specifically in HCT-116 cells.

The cytotoxicity effect of a crude ethanol extract of *S. macrophylla* seeds has demonstrated varying levels of cytotoxicity when screened against KB, Ca SKi, HCT 116, Hep G2 and MCF-7 cancer cell lines. Hence, the crude ethanol extract was further fractionated to yield hexane, ethyl acetate and aqueous fractions. The cytotoxicity effect was only observed in SMEAF treated cells and the lowest IC₅₀ value was against HCT116. Hexane and aqueous fractions were found to have no effect on all cell lines (IC₅₀ > 200 µg/ml in all cases). SMEAF did show higher values of IC₅₀ compared to ethanol extract especially on CasKi and MCF-7 but lower IC₅₀ values were obtained on KB, Hep G2 and HCT-116 cells. Eventually, the minimum IC₅₀ value of 35.5 µg/ml was obtained with the ethyl acetate fraction against HCT-116 cells. Thus, it can be concluded that ethyl acetate fraction was selectively toxic against HCT116 cells and hence further investigations were carried out using HCT116 cell line. To investigate the mechanism of cell death induced by SMEAF in HCT116 cells, flow cytometric analysis was done by PI and annexin V-FITC labeling to confirm apoptosis as a marker to assess apoptosis (Kawamura and Kasai, 2007). During apoptosis, a number of changes occur in cell surface markers that show affinity for PI and annexin V-FITC labeling (Vermees et al., 1995). The results in the present study indicated apoptotic population induced by the extract in a dose-and time-dependent manner. This was inferred on the basis of flow cytometric evidences, which showed an increment of apoptotic cell percentage in dose- and time-dependent manner by using Annexin V/PI staining. Another important characteristic of apoptosis induction is DNA fragmentation and measurement of DNA content makes it possible to identify apoptotic cells. TUNEL assay which measures DNA fragmentation or DNA strand breaks by the incorporation of Br-dUTP into the exposed 3'-OH DNA ends, followed by detection with fluorochrome-conjugated anti-BrdU antibody (Darzynkiewicz et al., 2008) was performed to further confirm the potential of SMEAF to induce apoptosis in HCT-116 cells.

The results which showed increasing TUNEL positive cells at 24 h, with increasing concentrations of SMEAF were found to be complementary with the results from Annexin V/PI. To recognize the cell cycle phase specificity and to quantify apoptosis, propidium iodide (PI) dye binds to DNA in cells at all stages of the cell cycle, and the intensity with which a cell nucleus emits fluorescent light is directly proportional to its DNA content.

Cell cycle analysis on a 24 h SMEAF treatment showed a significant increase in the percentage of G1-phase from 24.33% (control) to 55.34% of cells at the highest concentration of 1.0 mg/ml suggesting that SMEAF arrested the cells at G1-S transition in a dose-dependent manner. To further confirm the involvement of cell cycle arrest in apoptosis, cells were subjected to different periods of incubation of 24, 48 and 72 h. As depicted in Figure 4B,

there was a gradual increase in the percentage of Sub-G1 cells during prolonged induction by 0.05 mg/ml of SMEAF at 24, 48 and 72 h incubation periods. These results seem to suggest that the apoptosis occurred through G1 arrest in HCT-116. Generally, apoptosis is regulated through two distinct pathways: the cell surface death receptor and mitochondrial-mediated pathways (Reed, 2001; Desagher and Martinou, 2000) and the $\Delta\Psi_m$ loss of MMP appears to be a critical event in apoptotic process (Kroemer and Reed, 2000). In the present study, SMEAF treatment has induced $\Delta\Psi_m$ loss in the period of 24 h treatment performed by using JC-1 dye. The results demonstrated that concentration as low as 0.05 mg/ml of SMEAF was able to induce disruption of mitochondrial membrane integrity of tested HCT-116 cells up to 10.45%, while at the highest concentration of 1.0 mg/ml, 53.69% of cells were affected.

The investigation on intracellular total glutathione (GSH) level was performed to determine the involvement of oxidative stress in cell death. The depletion of GSH has been known to contribute to the onset of apoptosis, by rendering the cells more sensitive to apoptotic agents (Macho et al., 1997). Heales (1995) and Mithöfer and coworkers (1992) have reported the importance of GSH in maintaining optimum mitochondrial functions and the role of ATP production in maintaining GSH level, indicating the correlation of GSH to MMP. Thus, the deficiency in the energy supplied by mitochondria is likely to affect the cellular turnover of GSH that protects the cells from oxidative stress and consequently apoptosis. Treatment with SMEAF at 0.05mg/ml significantly depleted the level of GSH by as much as 49.56% (12.02 ± 0.59 nmoles) and showed ability in affecting the GSH level in a dose-dependent manner. Based on these results, it is believed that SMEAF might cause cancer cells to undergo oxidative stress leading to the depletion of GSH and disruption of the mitochondrial membrane integrity and eventually leading to apoptosis.

CONCLUSION

In conclusion, the present study highlights the anticancer and cytotoxic potential of seed extract of *S. macrophylla*. These results suggest that the cytotoxic activity of this plant has been due to its apoptosis inducing properties. This was evidenced by depletion of intracellular glutathione, disruption of mitochondrial membrane potential, DNA fragmentation, externalized phosphatidylserine and accumulation of sub-G1 population. Further studies to characterize the active principles and elucidate the mechanism of action are in progress.

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